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ENGINEERING EXPERIMENT STATION
OREGON STATE COLLEGE
CORVALLIS, OREGON

Bacterial Fermentation of Spent Sulfite Liquor for the Production of Protein Concentrate Animal Feed Supplement

By
Herman R. Amberg
West Coast Resident Engineer
National Council for Stream Improvement
(of the Pulp, Paper and Paperboard Industries), Inc.

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BACTERIAL FERMENTATION OF SPENT SULFITE LIQUOR FOR THE PRODUCTION OF PROTEIN CONCENTRATE ANIMAL FEED SUPPLEMENT

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SUMMARY AND CONCLUSIONS

A process for the treatment of spent sulfite liquor was developed by the National Council for Stream Improvement at the Oregon State College Engineering Experiment Station. The process, an aerobic fermentation, employed a mixed bacterial culture capable of reducing 80 to 90% of the biochemical oxygen demand (BOD) of spent sulfite liquor. Approximately 20,000 ppm of bacterial cells were carried in the fermentation unit, which was operated on a continuous flow basis. The fermentation was conducted to effect high BOD reductions and to produce high yields of bacterial protein having a protein content of 66 to 70%.

The results presented in this report warranted the following conclusions:

1. A bacterial culture was developed readily by inoculating diluted spent sulfite liquor with river water, or soil suspension. By continuous transfers, the concentration of cells was increased sufficiently to treat digester strength liquor.

2. The optimum fermentation temperature ranged from 30 to 40°C, and heat had to be added to maintain the fermentation temperature. By employing hot digester or hot steam-stripped liquor, it was possible to operate the process without the addition of heat.

3. The optimum pH was near neutrality. Steam stripping of the raw waste resulted in substantial savings in the neutralizing chemical requirements. The most suitable neutralizing agent was anhydrous ammonia. Using a steam-stripped spent sulfite liquor,

0.88 grams of ammonia were added per liter of digester liquor to increase the pH to 7.4. If calcium base liquor was used, the ammonia added to satisfy the nutrient requirements was sufficient to maintain the pH above 7.0.

4. When fermenting ammonia base liquor, the only nutrient added was phosphorus. Approximately 27% of the ammonia present in the ammonia base liquor was converted to bacterial protein. About 34 pounds of phosphorus (calculated as P) were added to the raw waste per ton of cells produced.

5. Both ammonia and phosphorus had to be added when treating calcium, magnesium, or sodium base sulfite liquors. About 302 pounds of anhydrous ammonia were added per ton of cells produced. The ammonia added as nitrogen was sufficient to neutralize the waste, and neutralization chemicals were not rerequired. The phosphorus requirements were the same regardless of the base.

6. Because of the high cell concentration carried in the aeration unit, mechanical aeration equipment was required. The oxygen uptake rate was 428 ppm per hour when 20,000 ppm of suspended cells were carried in the aeration unit. Employing a mechanical type aerator, which had an absorption efficiency of 23%, about 104 cubic feet of air were required per pound of BOD added. At a cell yield of 0.4 pound per pound of BOD added, 260 cubic feet of air had to be supplied per pound of cells produced, or 0.045 horsepower per pound of cells produced.

7. The bacterial cells were readily separated from the treated waste by sedimentation or centrifugal separation. The volume of the cell suspension was reduced 50% by conventional settling. With an influent to the clarifier of 2% cell suspension, the thickened liquor contained 4% cells. Cell concentrations in excess of 4% were not attainable by sedimentation. Approximately 24-hour storage capacity for the cells would have to be incorporated into the design of the clarifier.

8. At least 2 or 3 washings were required to remove the fermentation broth from the cells. Washing was conducted with centrifuges.

9. The process was successfully operated at cell concentrations ranging from 9000 to 32,000 ppm, and using waste concentrations ranging from 0.4 to 8.2% total solids. It was possible by sulfur dioxide removal to treat 12% strength liquor having an average BOD of 39,000 ppm.

10. The optimum BOD loading when using SO_2-stripped digester strength liquor having a BOD of 39,000 ppm, was 2600 pounds per 1000 cubic feet of aeration capacity per day. The BOD reduction at this load was 84%.

11. The optimum washed cell yield was 0.4 pound per pound of BOD added. At a collection efficiency at the blowpits of 500 pounds of BOD per ton of pulp, 200 pounds of cells containing 68% protein would be produced per ton of pulp.

12. The cells contained 66 to 70% protein (depending upon the washing efficiency), 6 to 8% ash, 1.5% phosphorus, and 0.42% calcium. The cell material contained most of the essential vitamins and amino acids in high concentrations.

13. Preliminary feeding studies on rats indicated the cell material was palatable, nontoxic, and an excellent protein supplement.

14. The operating costs on fermenting ammonia base liquor were $55.62 per ton of 66% protein product, and the total product cost was $81.20. Bacterial cells could be produced at a profit for $120 per ton of 66% product.

I. INTRODUCTION

An investigation was initiated at the Engineering Experiment Station at Oregon State College to study the feasibility of reducing the BOD of spent sulfite liquor by biological treatment.

Numerous attempts have been made to treat spent sulfite liquor by conventional sewage treatment methods (1, 3, 5, 7, 9). Generally speaking, however, the use of conventional secondary sewage treatment devices would necessitate the construction of a plant which would dwarf the pulp mill. Because of the pollutional strength of spent sulfite liquor, it would be economically unsound to apply sewage treatment methods to the treatment of this waste.

Spent sulfite liquor has been used successfully as a substrate in the fermentation industry, where a number of pure culture fermentations have been used to partially reduce the BOD of the waste (4, 6). In general, the BOD reduction by fermentation, using pure cultures of yeast, could be expected to range from 50 to 65%.

It was thought that by incorporating some of the methods used in the fermentation industry and in the sewage treatment field, a method of treatment might be developed which would effect high BOD reductions. It was decided that a mixed culture of bacteria would be more effective in reducing the BOD of the waste than pure cultures of microorganisms, and that an extremely heavy inoculum or cell concentration would have to be carried in the aeration unit.

Preliminary experimentation with the aerobic fermentation process, using a mixed bacterial culture, indicated the waste could be treated readily, but the air and chemical requirements would be high, and disposal of cells would become a problem.

An analysis of the cell material indicated the washed cells contained about 66 to 70% crude protein. Because of the high protein content and the relatively high cell yield, it was decided to use the cell material as a high protein supplement in the diet of animals.

In the Pacific Coast area, approximately 360,000 tons of oilseed meals are consumed annually by livestock--most of the material being shipped in from the Midwest. If high protein feed could be produced on the West Coast at a cost which would enable it to compete with oilseed meals now used, and if feeding tests prove the cell material to be equal nutritionally to its potential, an adequate market would be available. Through the sale of a byproduct it would be possible to defray the cost of treatment and ultimately operate at a profit.

This paper presents the operating results on the aerobic fermentation of spent sulfite liquor as determined from a semi-pilot plant operating on a continuous flow basis. Most of the experiments were conducted using raw waste containing substantial amounts of free SO_2, but one experiment using SO_2-free waste was conducted and the results recorded.

A complete analysis of the bacterial cell protein concentrate also is included in the report, as well as some preliminary rat feeding studies.

II. EXPERIMENTAL METHODS AND EQUIPMENT

A schematic diagram of the apparatus is shown in Figure 1. Raw, spent sulfite liquor was pumped by a constant displacement pump from a 55-gallon storage tank to the aeration tank, where it was aerated in the presence of bacterial cells. The aerator was heated to the desired temperature by means of a stainless steel immersion heater controlled by a thermostat. The aeration volume was maintained at 48.8 liters and the fermentation temperature was maintained at 34 to 36 °C. The mixed aeration tank liquor flowed by gravity to a conical settling basin (50-liter capacity), where the cells were removed from the supernatant liquor. The effluent passed over the overflow weir of the clarifier and was discharged to the sewer. The settled bacterial cells were pumped back to the aeration chamber. The cells which accumulated in the clarifier were withdrawn once a day to maintain a constant concentration of cells in the aeration unit.

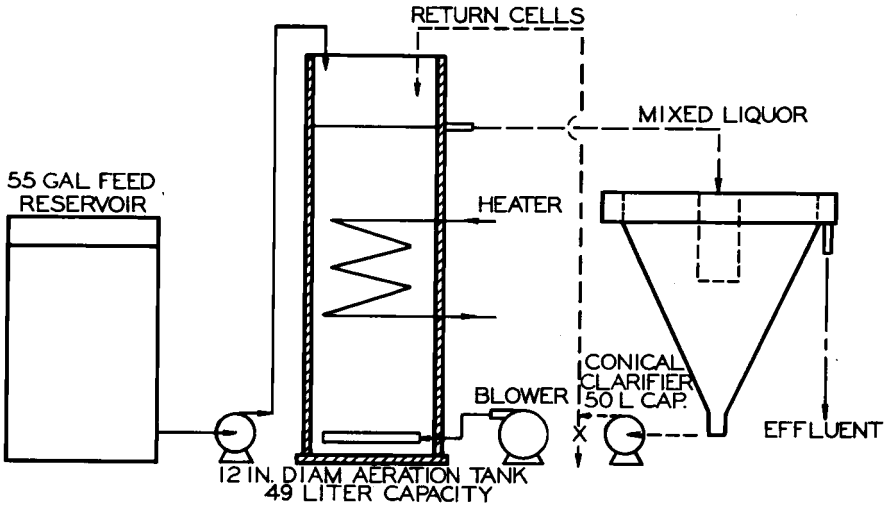

Figure 1. Diagram of semipilot plant for aerobic fermentation of spent sulfite liquor.

In the studies reported, ammonia base liquor was used. The ammonia base liquor, obtained as digester liquor of 12% solids and a BOD ranging from 30,000 to 42,000 ppm, was diluted with tap water to give the desired concentration. The raw waste concentration was varied from 0.4 to 8.2% solids. The waste was neutralized to pH 7.0 to 7.4 with sodium carbonate and supplemented with phosphorus to insure a BOD to phosphorus ratio of 80 to 1. Dow Corning Anti-Foam AF emulsion was added to the raw feed to reduce foaming.

Daily BOD determinations were conducted on both effluent and raw waste. The cell concentration in the aeration unit was checked daily by suspended solids determination, employing the Gooch method (asbestos mat). The cell yield was determined daily by suspended solids determinations. The aeration tank mixed liquor, effluent, and raw waste received daily pH checks.

The cells used in the feeding studies were washed 5 or 6 times with tap water to remove the spent liquor. The cell material was dried at 70°C and used for the animal feeding studies.

The following are some of the terms used and detailed descriptions of the methods involved:

1. BOD--conducted daily on raw waste and effluent using Standard Method dilution water. The water was seeded with bacteria obtained from the oxidation plant.

2. Suspended solids determinations--conducted daily on aeration tank liquor and excess cells. A 10-milliliter sample was prediluted 10 times and 5 milliliters of the prediluted cell suspension were added to a weighed Gooch. The cells were washed 3 times in the Gooch and dried at 105°C for 24 hours.

3. Cell yield--determined daily by conducting suspended solids determinations. The washed cell concentration, multiplied by the volume of cells wasted, was recorded as cell build-up.

Example:

Cell concentration,	37.0 grams/liter
Volume of cells wasted,	18 liters
Cell build-up = 37.0 × 18.0 =	666 grams

4. Protein--content of cells determined on each experimental run. The cells were washed 6 times and centrifuged to remove the spent liquor, then dried at 105°C. Duplicate 2.0-gram samples were used to determine the Kjeldahl nitrogen. The nitrogen concentration multiplied by 6.25 was recorded as percent protein.

5. Loading parameters--expressed by the following:

   a) Pounds of BOD/cu ft of aeration capacity/day --BOD load in pounds fed to a cubic foot of aeration capacity per day.

   b) Pounds of BOD/lb of cells/day--BOD load applied per pound of cells carried in the aeration unit.

   c) Retention--time of waste retention in aeration unit expressed in hours.

6. Cell age--time in hours cells remained in aeration unit, or amount of cells in aeration unit in grams divided by the quantity of cells in grams wasted per day.

From 3 to 5 days were allowed to permit the system to come to equilibrium, and each experiment was conducted for 6 to 12 days after equilibrium was established. A run averaging 6 or more days was sufficient to obtain reliable data on cell yield and BOD reductions.

III. RESULTS AND DISCUSSION

Bacterial culture developed

The bacterial culture was started by inoculating weak, spent sulfite liquor with river water and allowing the bacteria to increase in numbers by continually returning the excess cells. As the bacteria increased in numbers, the waste concentration fed was increased proportionately.

Microscopic study of the organisms present in the aeration tank indicated that the population was entirely bacterial in nature. No protozoa, yeast, or filamentous organisms were present.

The predominant culture consisted of Gram negative rods of the Pseudomonas group. A Gram negative rod, which was a true thermophile and extremely active at 55°C, also was isolated. The total viable count obtained from one milliliter of diluted cells at various temperatures was as follows:

Temperature, °C	Count/ml
22	450,000
30	8,500,000
35	9,000,000
55	360,000

The above summary clearly indicated that the cultures employed had an optimum temperature at 30 to 35°C, and growth decreased considerably at 22 and 55°C. The biological oxidation could be carried out at 30°C instead of 34°C without an appreciable decrease in efficiency. The presence of a true thermophile indicated the possibility of a thermophilic bio-oxidation. Cursory experimentation in the thermophilic range indicated the feasibility of operation at the elevated temperature.

The cells in the aeration unit resembled a clay suspension. Upon dilution with supernatant liquor or wash water, the cells coalesced to form floc, which settled rapidly. The washed cells were light brown in color and were tasteless when dried.

Washed cells contained 4 to 8% ash and 66 to 74% protein. Protein and ash contents varied somewhat with the degree of washing. The average protein concentration of washed cells was about 66 to 70%.

Reaction

Optimum biological oxidation occurred near neutrality. The ammonia base digester liquor used in the experiments had a pH of 3.0 to 3.4. The neutralization data for sodium carbonate, lime (CaO), sodium hydroxide, and ammonium hydroxide are presented in Table 1.

The effect of steam stripping of spent liquor upon the alkali requirement is shown in Tables 2 and 3 for ammonium hydroxide and sodium hydroxide. Considerable savings in alkali were effected by steam stripping the raw liquor prior to neutralization.

Tables 4 and 5 clearly show the savings in neutralizing chemicals resulting from removal of SO_2. Chemical requirements were cut in half by stripping the raw liquor to pH 4.70.

The cheapest chemical is lime--costing about $3.10 per ton of cells (70% protein) produced. If the liquor is further processed for lignin recovery, however, lime would cause serious difficulties in processing. Where calcium base pulping is used, ammonia added to satisfy the nitrogen requirements would be more than adequate to maintain a pH above 7.0. For example, when using CaO base liquor, about 27 to 39 pounds of anhydrous ammonia will have to be added per ton of pulp, which is in excess of the neutralization requirements.

In the neutralization of ammonia base liquor, lime would be the cheapest chemical and anhydrous ammonia would be second at a cost of $7.40 per ton of cells for stripped liquor. Lime also could be used to precipitate SO_2 as calcium sulfite, thereby eliminating the stripping operation. Using lime to remove SO_2 would necessitate an additional sedimentation tank for the removal of precipitated fines and sludge, resulting in loss of recoverable SO_2 (about $0.50/ton of pulp). The use of lime also results in precipitation of the phosphorus as calcium phosphate.

Table 1.

Spent sulfite liquor neutralization data

(Digester strength liquor-- 12% solids)

pH	Grams of alkali added per liter of liquor			
	Sodium carbonate	CaO	NaOH	NH_3
3.10*	0.0	0.0	--	--
3.40**	--	--	0.0	0.0
4.00	1.6	0.8	0.6	0.3
5.00	3.7	1.8	2.1	0.9
6.00	5.9	2.8	3.1	1.4
7.00	8.6	3.8	3.7	1.7
8.00	14.4	5.0	4.2	2.1
9.00	28.0	7.8	--	--

* Sample had initial pH of 3.10. Used for Na_2CO_3 and CaO neutralization curves.

** Sample had initial pH of 3.40. Used for NaOH and NH_4OH neutralization curves.

Table 2.

Effect of steam stripping upon alkali demand of digester strength ammonia base liquor

(12% solids content)

pH \ pH of stripped liquor	3.40	3.90	4.10	4.50	4.70
	Grams of ammonia per liter of liquor				
4.0	0.3	--	--	--	--
5.0	0.9	0.7	0.5	0.3	0.2
6.0	1.4	1.2	1.1	0.7	0.6
7.0	1.7	1.4	1.3	0.9	0.8
8.0	2.1	1.8	1.5	1.2	1.0

Table 3.
Effect of steam stripping upon alkali
demand of digester strength ammonia base liquor
(12% solids content)

pH / pH of stripped liquor	3.40	4.10	4.50	4.70
	Grams of NaOH per liter of liquor			
4.0	0.6	--	--	--
5.0	2.1	1.2	0.7	0.5
6.0	3.1	2.0	1.4	1.2
7.0	3.7	2.5	1.8	1.5
8.0	4.2	3.4	2.5	2.2

Table 4.
Alkali requirements to neutralize
digester strength ammonia base liquor to pH 7.4

Chemical	Unstripped liquor gm/liter	Stripped liquor gm/liter	Lb of alkali/ton of pulp	
			Unstripped liquor	Stripped liquor
Sodium carbonate	11.00	--	192	--
CaO	4.00	1.80	70	31.5
NaOH	4.00	1.63	70	28.6
NH_3	1.85	0.88	32	15.4

Table 5.
Cost of alkali required to
neutralize digester strength liquor to pH 7.4

Chemical	Cost/ton of pulp, $		Cost/ton of cells, $	
	Unstripped liquor	Stripped liquor	Stripped liquor	
			70% protein	50% protein
CaO ($20/ton)	0.70	0.31	3.10	2.21
NaOH ($90/ton)	3.15	1.29	12.90	9.21
NH_3 ($96/ton)	1.54	0.74	7.40	5.28

Nutrients

1. Nitrogen. Since ammonia base spent sulfite liquor was used in these studies, an ample supply of nitrogen was present in the waste to meet the needs of the bacterial culture. If calcium, magnesium, or sodium base liquors were used to produce bacterial protein concentrate, a source of nitrogen would have to be added. The cheapest form of nitrogen was anhydrous ammonia, which was selling for approximately $96 per ton.

The conversion of ammonia to bacterial cell material is easily calculated. In ammonia base pulping, approximately 100 pounds of ammonia are added per ton of pulp produced, or 82.4 pounds of nitrogen. At the optimum protein content of 70%, about 224 pounds of nitrogen are incorporated in a ton of bacterial cells. At a yield of one-tenth of a ton of cells per ton of pulp, approximately 27.2% of the nitrogen present in the waste is converted to organic nitrogen.

When using calcium base liquor, a minimum of 22.4 pounds of nitrogen will have to be added to the spent liquor from a ton of pulp, assuming 100% utilization efficiency. Since such high efficiencies are impossible in actual practice, an absorption efficiency of 90% is assumed. At 90% efficiency, 24.9 pounds of nitrogen or 30.2 pounds of anhydrous ammonia will have to be added per ton of pulp. A 200-ton calcium base mill would then require 6040 pounds of ammonia per operating day. At $0.05 per pound, the cost of anhydrous ammonia is $15.10 per ton of cells produced (65% protein).

Further experimentation may show that the nitrogen requirements can be reduced substantially through higher utilization efficiencies. At a utilization efficiency of 100%, 27.2 pounds of ammonia will be required per ton of pulp, and the cost per ton of cells (65% protein) would then be $13.95.

The saving effected by recovery of about 27% of the nitrogen used in ammonia base pulping, plus the savings in somewhat higher pulp yields, gives ammonia base pulping an economic advantage over the calcium base process if fermentation of spent liquor is desired. The use of the soluble base would facilitate further processing of the spent broth for lignin recovery.

2. Phosphorus. A sample of bone dry bacterial cells, which were washed to a protein content of 64%, contained 1.52% phosphorus (calculated as P). Assuming 100% efficiency of phosphorus utilization, 0.0152 pound of phosphate was required per pound of cells produced, or 30.4 pounds per ton of cells. At a utilization efficiency of 90%, the phosphorus requirements were then 33.8 pounds of phosphorus per ton of cells. The cheapest form of phosphorus was phosphoric acid ($6.70 per 100 pounds--75%), but additional neutralization chemicals also were required. The phosphorus requirements per ton of cells cost $9.54, and the additional ammonia requirements for pH control cost $0.90, which made a total cost of $10.44 per ton of cells for the phosphorus addition.

3. Calcium. The raw waste contained sufficient quantities of calcium to ensure optimum biological oxidation. However, the Ca to P ratio in the protein concentrate was of importance in feeding poultry and pigs. An analysis of the cells indicated that the calcium content of the cells was 0.42% (calculated as Ca). The 64% protein content sample then had a calcium to phosphorus ratio of 0.42/1.52, or 0.276, which was insufficient to prevent the development of rachitogenic disorders (4). Adjustment of the Ca to P ratio could have been brought about by the addition of calcium salts to the diet of poultry or pigs.

Oxygen uptake rates of cells

Oxygen uptake rates of the bacterial cells were determined by using a Warburg respirometer. Three spent sulfite liquor loadings were used in these studies: 0.5, 0.75, and 1.0 grams BOD per gram of cells.

The raw waste was neutralized with sodium carbonate to pH 7.2, and phosphorus was added as phosphoric acid. All of the flasks were incubated at 31°C for 2 hours, and oxygen uptake readings were recorded at 15-minute intervals.

The oxygen uptake of the cells is presented in Figure 2, and a summary of the oxygen uptake experiments is presented in Table 6.

Table 6.
Summary of oxygen uptake studies

Flask no.	Cells per flask, gm	Spent sulfite liquor added gm of BOD	BOD load gm BOD per gm of cells	Oxygen uptake μL/hr	QO_2 μL/gm/hr
1	0.102	--	--	470	4,608
2	0.051	0.0255	0.500	610	12,000
3	0.041	0.0306	0.749	550	13,400
4	0.034	0.0340	1.000	510	15,000

The oxygen uptake rate, or QO_2, for the cells alone was 4610 μL per gram of cells per hour. An increase in the QO_2 values was noted with increased BOD loadings, and at the optimum loading of 1.0 gram BOD per gram of cells, the QO_2 was 15,000 μL per gram of cells per hour.

At the upper loading of 1.0 gram of BOD per gram of cells, the uptake rate was 21.4 milligrams of oxygen per gram of cells per hour. The amount of oxygen necessary to supply a 30,000-ppm culture was 642 ppm per hour, or for a 20,000-ppm culture, 428 ppm per hour.

At a BOD load of 1.5 pounds per cubic foot of aeration capacity per day, and with 20,000 ppm of cells in the aeration unit, 4.8 cubic feet of oxygen were required for every pound of BOD added, or 24 cubic feet of air (100% absorption efficiency). Employing a mechanical type aerator having an absorption efficiency of 23%, about 104 cubic feet of air were required per pound of BOD added. At a cell yield of 0.4 pound per pound of BOD added, 260 cubic feet of air were supplied per pound of cells produced.

The power requirements when using the mechanical aerator were about 0.045 horsepower per pound of cells produced. At a power cost of $0.005 per kwhr, the power cost per ton of cells produced was $8.05 (65% protein content). The power cost based on a ton of product having a protein content of 50% would be $6.19.

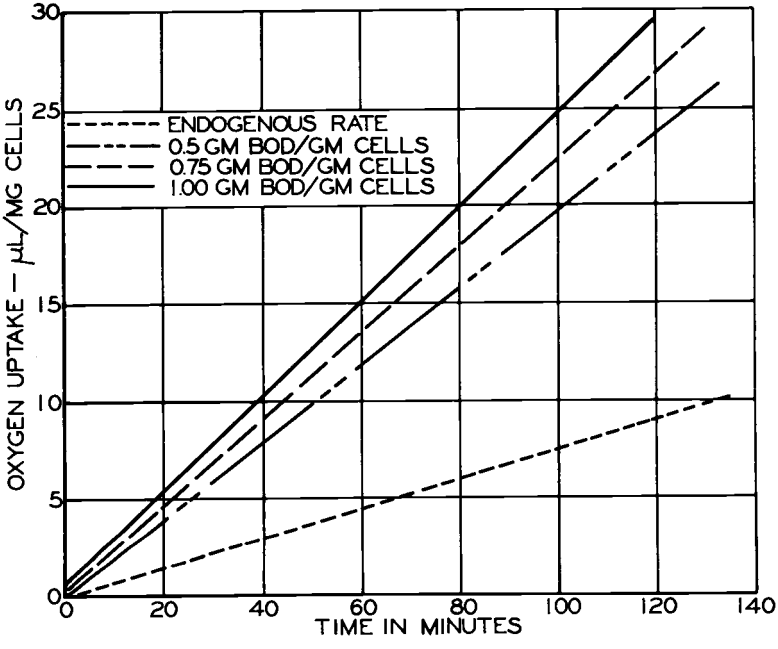

Figure 2. Oxygen uptake rate of bacterial cells.

Sedimentation rates of bacterial cells

Sedimentation tests were conducted on two samples of mixed liquor from the aeration unit. The samples had an initial cell concentration of 19.2 and 20.0 grams per liter. The tests were conducted at room temperature using 1000 milliliter graduates as settling tanks. The cells were diluted with tap water to give initial cell concentrations ranging from 4.0 to 19.2 grams per liter.

The initial cell concentration in each of the 10 tests is given in Table 7. The results of the sedimentation tests are given in Tables 8 and 9. The cell volume, as well as the cell concentration, are given in the tables. The solids concentrations tabulated refer only to the cells assuming no extraneous material. For example, the total solids content of a 1.92% cell suspension would run about 11.0%.

Table 7.
Cell concentrations at start of settling test

	1	2	3	4	5	6	7	8	9	10
Cells, ml	1000	900	800	700	600	600	500	400	300	200
Water, ml	0	100	200	300	400	400	500	600	700	800
Total vol, ml	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000
Susp solids, %	1.92	1.73	1.54	1.34	1.15	1.20	1.00	0.80	0.60	0.40
Cell concentration, gm/ℓ	19.2	17.3	15.4	13.4	11.5	12.0	10.0	8.0	6.0	4.0

Table 8.

Settling characteristics of bacterial cells

Time in hr	1		2		3		4		5	
	Volume ml	Solids %	Volume ml	Solids %	Volume ml	Solids %	Volume ml	Solids %	Volume ml	Solids %
0	1000	1.92	1000	1.73	1000	1.54	1000	1.34	1000	1.15
5.7	975	1.97	963	1.80	950	1.62	920	1.46	470	2.45
18.0	930	2.06	900	1.92	840	1.83	488	2.75	370	3.11
20.7	920	2.09	880	1.97	820	1.87	470	2.85	360	3.20
23.7	912	2.11	868	2.00	800	1.93	455	2.95	348	3.31
30.2	890	2.16	830	2.08	755	2.04	430	3.12	330	3.49
44.0	847	2.27	768	2.26	670	2.30	392	3.42	300	3.84
48.0	835	2.30	753	2.30	650	2.37	385	3.48	295	3.90

Table 9.

Settling characteristics of bacterial cells

	6		7		8		9		10	
0	1000	1.2	1000	1.0	1000	0.8	1000	0.6	1000	0.4
1.0	978	1.2	620	1.6	520	1.5	405	1.5	270	1.5
2.0	860	1.4	500	2.0	450	1.8	350	1.7	230	1.7
3.0	740	1.6	470	2.1	420	1.9	320	1.9	210	1.9
5.5	600	2.0	420	2.4	365	2.2	280	2.1	180	2.2
18.5	460	2.6	330	3.0	270	3.0	195	3.1	120	3.3
21.0	445	2.7	325	3.1	260	3.1	185	3.2	118	3.4
24.4	430	2.8	315	3.2	250	3.2	180	3.3	112	3.6
30.0	402	3.0	300	3.3	230	3.5	170	3.5	106	3.8
43.7	366	3.3	270	3.7	208	3.8	150	4.0	98	4.1
48.0	358	3.4	267	3.7	200	4.0	148	4.1	95	4.2

A summary of Tables 8 and 9, presented in Table 10, gives the 24- and 28-hour cell concentrations. The highest cell concentration was attained when the initial cell concentration was about 1% or 10 grams per liter. The 2% cell suspension did not thicken in periods up to 48 hours. This did not mean, however, that the 2% mixed liquor would not thicken. For example, in the laboratory semipilot unit a waste rentention of 4 to 6 hours was employed with a cell thickening period of 24 hours, and the average concentrations of cells withdrawn ranged consistently from 3.5 to 4.0%.

As the mixed liquor passed through the supernatant liquor in the thickener it was diluted, and coagulation or floc formation of the cells occurred. The coagulated cells settled rapidly and were allowed to compact for 24 hours. At the end of 24 hours the cells occupied about 30 to 40% of the thickener volume. The side water depth in the laboratory thickener was only 22 inches, and with additional hydrostatic head in actual practice, the cells would be expected to thicken to at least 4%. The total solids concentration of the thickened liquor leaving the thickener would be about 13% when using a spent sulfite liquor of about 8.2% solids.

In the design of a thickener, sufficient capacity must be supplied for cell storage of about 24 hours. Allowance of approximately 50% of the thickener capacity for cell storage should be adequate to produce a thickened cell suspension containing about 40 grams of cells per liter of suspension.

No difficulty was encountered in the laboratory in the decomposition of the cells when employing storage periods in excess of 48 hours. The cells appeared to be quite stable. Some hydrogen sulfide production occurred in the laboratory thickener, but this was eliminated by thoroughly cleaning the thickener at weekly intervals. If continuous scraping mechanisms were employed for the removal of the cells, it would be doubtful if an odor problem would occur. To confirm these results, however, a pilot plant study would be required.

Table 10.
Summary of sedimentation tests

Initial cell concentration at start of test	Cell concentration after 24-hour sedimentation %	Cell concentration after 48-hour sedimentation %
1.92	2.11	2.30
1.73	2.00	2.30
1.54	1.93	2.37
1.34	2.95	3.48
1.15	3.31	3.90
1.20	2.80	3.40
1.00	3.20	3.70
0.80	3.20	4.00
0.60	3.30	4.10
0.40	3.60	4.20

Centrifuge studies

To arrive at an approximation of the thickening characteristics of bacterial cell material, a series of centrifugal separation tests were conducted using the mixed liquor from the aeration unit. It was assumed the entire thickening operation would be conducted by centrifugal separators.

For the centrifuge studies, an International, size 2 centrifuge (batch type) was used with 250-milliliter cups. Operation was conducted at 900 gravities. The effect of time of centrifugal separation upon cell concentration and solids content of the centrifuged product is shown in Table 11 and Figure 3. In five minutes of centrifuging at 900 gravities, a 63% reduction in volume was accomplished. The resultant thickened cells had a total solids content of 13.1%. About 46% of the total solids were cell material, and when dried the centrifuged product had a protein content of 32%. By successive centrifuging it was possible to reduce the initial volume by 80%, thereby producing a product having a protein content, when dried, of 41% and total solids concentration of 18.6%.

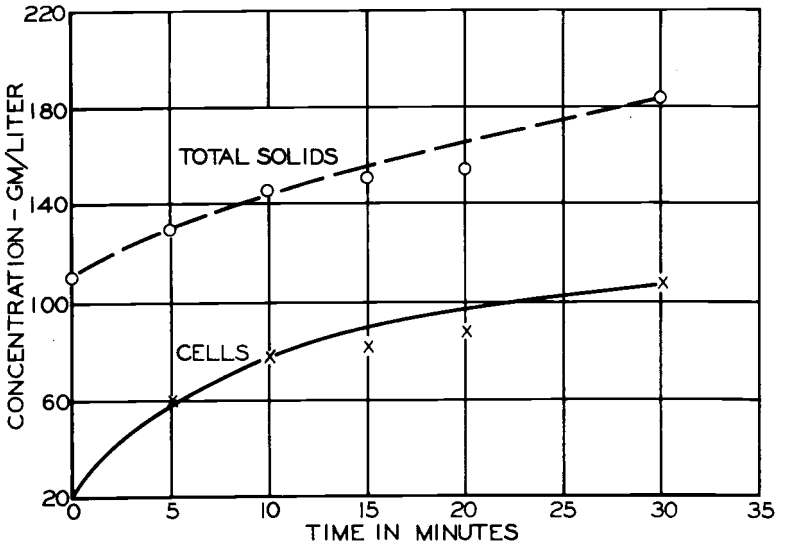

Figure 3 Effect of time of centrifugal separation upon solids concentration (900 × G)

Table 11.
Centrifugal separation tests
(900 × gravity)

Time of separation min	Cell concentration gm/ℓ	Total solids of cell suspension gm/ℓ	Cells in product %	Protein of dried product %	Volume reduction %
0	22.0	111.0	19.8	--	--
5	59.8	131.0	45.6	31.9	63.2
10	78.1	146.0	53.5	37.4	71.8
15	82.0	148.3	55.2	38.6	73.2
20	88.0	152.3	57.7	40.4	75.0
30	110.0	186.0	59.2	41.4	80.0

It was not possible, without multiple washing of cells, to produce a product having a protein content of 50% or more. The protein content desired determined the number of washings and the

solids content of the final product. For some purposes, one washing operation would suffice, but two or three washings would bring down to well under 1% the concentration of spent liquor solids.

Cell yield was influenced by the degree of washing or by the protein content of the desired product. At a protein content of 50%, about 280 pounds of bone dry cell material would be produced per ton of pulp, and at a protein content of 60%, the yield would be 230 pounds per ton of pulp. If a product having a 50% protein content was desired, one washing followed by further centrifugal thickening would produce a cell suspension having a total solids content in excess of 15%.

The relationship between cell yield and protein content is presented in Figure 4.

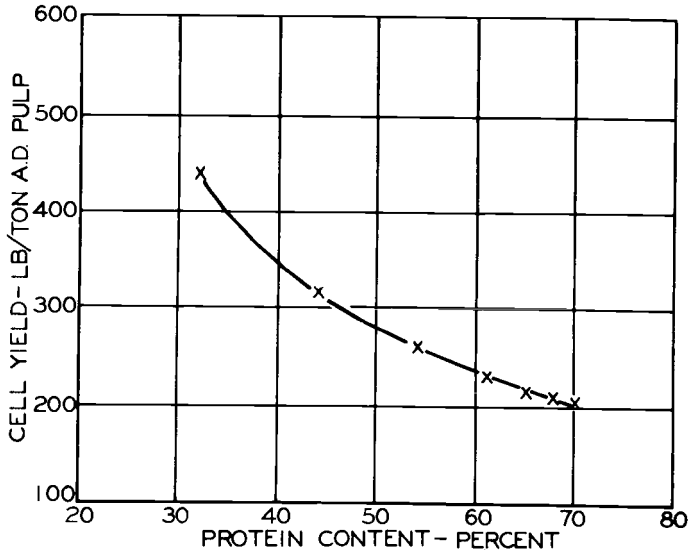

Figure 4. Relationship between protein content and cell yield.

Operational data

A total of 11 runs are reported and summarized in Table 12.

All of the operational data were recorded after equilibrium conditions were attained. Usually 4 to 5 days were allowed to permit the system to come to equilibrium. Waste strength varied from a BOD of 1070 ppm to a maximum of 20,700. The strength of raw waste used over the range of 0.4 to 8.2% total solids did not materially affect efficiency, provided the cell concentration was increased proportionately. Some operational difficulty was observed when feeding spent liquor having a solids concentration of 9.0%. The difficulty was caused by excessive concentrations of SO_2, and removal of SO_2 permitted much higher loadings upon the aeration system.

The cell concentration in the aeration unit was varied from 8900 ppm to a maximum of 32,500 ppm. At the upper concentration, a BOD load of 1600 to 2100 pounds per 1000 cubic feet of aeration capacity per day was applied to the system, with resultant BOD reductions of 84 to 86%. The optimum cell yield at cell concentrations of 30,000 ppm was about 0.3 pound per pound of BOD added, or 0.36 pound per pound of BOD removed. When the cell concentration in the aeration unit was decreased to 21,000 ppm, the cell yield increased to 0.4 pound per pound of BOD added.

Table 12.
Summary of operational results on biological oxidation
of spent sulfite liquor for the production of high protein feed

	Run number					
	1	2	3	4	5	6
Duration of run after equilibrium, days	9	7	8	8	12	9
Feed rate, liters/hr	8.95	9.20	4.33	4.33	4.33	1.73
BOD of raw waste, ppm	1,070	2,000	3,150	4,460	5,841	16,400
pH of raw feed	7.00	7.20	7.20	7.30	7.20	7.40
BOD load, lb/1000 cu ft/day	295	562	420	593	776	871
BOD load, lb/lb of cells/day	0.912	0.752	0.521	0.642	0.594	0.502
Retention in aerator, hr	5.45	5.31	11.26	11.26	11.26	28.20
Retention in clarifier, hr	2.74	2.76	3.43	3.43	3.43	2.90
Avg suspended cells in aerator, ppm	8,940	11,980	12,910	14,820	20,962	31,200
Washed cell yield, lb/lb of BOD added	0.12	0.157	0.263	0.265	0.264	0.342
Washed cell yield, lb/lb of BOD removed	0.139	0.193	0.325	0.339	0.338	0.448
Cell age, days	12.2	8.5	7.3	5.9	6.4	6.6
Protein content of cells, %	--	--	--	--	--	66.0
Ash content of cells, %	5.5	5.0	5.2	5.0	5.0	4.0
BOD of effluent, ppm	125	372	600	970	1,285	3,910
BOD reduction, %	88.3	81.4	81.0	78.2	78.1	76.3

Table 12 (cont'd).
Summary of operational results on biological oxidation
of spent sulfite liquor for the production of high protein feed

	Run number				
	7	8	9	10	11
Duration of run after equilibrium, days	11	11	10	6	11
Feed rate, liters/hr	3.02	3.86	2.93	3.41	2.54
BOD of raw waste, ppm	17,225	17,000	17,600	19,410	20,700
pH of raw feed	7.40	7.20	7.20	7.20	7.20
BOD load, lb/1000 cu ft/day	1,600	2,010	1,575	2,030	1,610
BOD load, lb/lb of cells/day	0.787	1.06	1.64	1.17	1.22
Retention in aerator, hr	16.2	12.6	16.7	14.3	19.2
Retention in clarifier, hr	2.70	2.40	--	--	--
Avg suspended cells in aerator, ppm	32,460	30,400	26,600	27,800	21,100
Washed cell yield, lb/lb BOD added	0.301	0.306	0.354	0.348	0.400
Washed cell yield, lb/lb BOD removed	0.360	0.356	0.450	0.425	0.500
Cell age, days	4.2	3.1	2.74	2.28	2.06
Protein content of cells, %	70.4	74.2	70.62	67.00	65.40
Ash content of cells, %	4.0	4.0	5.0	6.0	7.0
BOD of effluent, ppm	2,820	2,450	2,560	3,583	4,130
BOD reduction, %	83.6	85.6	85.5	81.6	79.5

Effect of SO_2 removal upon treatment efficiency

In previous experiments it was found that when the raw waste concentration exceeded 8.5%, failure of the treatment process resulted. It was thought the high concentration of SO_2 might appreciably affect the treatment process.

A preliminary experiment was conducted in which digester strength liquor of 12% solids and with an average BOD of 39,000 ppm was used as the raw feed. Pretreatment of the raw waste with CaO resulted in precipitation of the SO_2 as calcium sulfite. It was found that when the SO_2 was removed, digester strength liquor could be fed to the aeration unit without any predilution. One of the difficulties encountered with the use of lime for SO_2 removal was the precipitation of the phosphorus used as a nutrient. If SO_2 was removed by steam stripping, phosphate precipitation would not occur.

A summary of the preliminary experiment using SO_2-free raw waste, and another experiment in which unstripped waste was used, is presented in Table 13.

It became apparent that SO_2 removal would be beneficial in treating the spent sulfite liquor because, when using SO_2-free waste, higher BOD reductions would result at almost twice the BOD loadings.

Table 13.
Comparison of treatment efficiency
using SO_2-free waste and untreated waste

	SO_2-free waste*	Unstripped waste
BOD of raw waste, ppm	39,000	20,700
Total solids of waste, %	12.0	8.2
Cells in aerator, ppm	22,000	21,100
Retention in aerator, hours	22.6	19.2
BOD load, lb/1000 cu ft/day	2,580	1,610
BOD of effluent, ppm	6,100	4,130
BOD reduction, %	84	80

*SO_2 was removed by lime precipitation.

Cell yields were not available for the experiment on SO_2-free waste because of the build-up of the precipitated phosphate, which made a solids balance impossible. Further studies should be conducted using steam-stripped waste. It appeared that cell yields of 0.5 pound per pound of BOD applied would be possible when using steam-stripped waste.

Cell yield

Referring to Table 12, it can be seen that the cell yield varies from a low of 0.12 to a high value of 0.4 pound per pound of BOD added. To explain this variation, the fundamental reactions occurring in the aerator must be studied. There are three reactions occurring simultaneously, as follows:

Organic matter oxidation

$$\text{Organic matter} + O_2 \longrightarrow CO_2 + H_2O \qquad (1)$$

Cell synthesis

$$\text{Organic matter} + O_2 + NH_3 \longrightarrow \text{bacterial cells} + CO_2 + H_2O \qquad (2)$$

Cell material oxidation (endogenous respiration)

$$\text{Bacterial cells} + O_2 \longrightarrow CO_2 + H_2O + NH_3 \qquad (3)$$

Approximately 50% of the oxidizable organic material is oxidized to gain energy for the growth of cells (equation 1), and the remaining 45 to 50% of the organic nutrients are assimilated into cell substance (equation 2). If the BOD loading to the aeration unit is sufficiently low, the major portion of the bacterial cells are oxidized (equation 3), resulting in low cell yields. To obtain high BOD reductions and high cell yields, reactions 1 and 2 should be favored and equation 3 should be reduced to a minimum.

It will be noted that at extremely low BOD loadings low cell yields occurred, but at higher BOD loadings the cell yield

increased substantially, with an optimum yield of 0.4 pound per pound of BOD added.

The optimum cell yield obtained in the experiments was 0.4 pound per pound of BOD added, or 0.5 pound per pound of BOD removed. Whether or not this yield could be increased by stripping the waste of SO_2 was questionable, although the load and BOD reduction could be substantially increased. For example, if 50% of the oxidizable organic material was oxidized to meet the energy requirements of the cells, then the cell yield could not exceed 0.5 pound per pound of BOD removed.

The cell yield was also influenced by the washing efficiency, which subsequently affected the protein content. Complete washing to free the cells of spent sulfite liquor would result in a yield of 0.4 pound of cells per pound of BOD added, and contain 68 to 70% protein. By decreasing the washing efficiency, the cell yield would increase, with a subsequent decrease in the protein content of the cells. For example, if it was desired to obtain a product containing 70% protein, the yield would then be 200 pounds per ton of pulp. However, if the degree of washing was decreased to produce a product containing 50% protein, the yield would then be 280 pounds per ton of pulp.

Figure 5 presents the cell yields as a function of BOD collected, and also the protein content of the cells. There was considerable variability in waste strength, but on the average about 500 pounds of BOD could be collected per ton of pulp produced (assuming 80% solids collection at the pits).

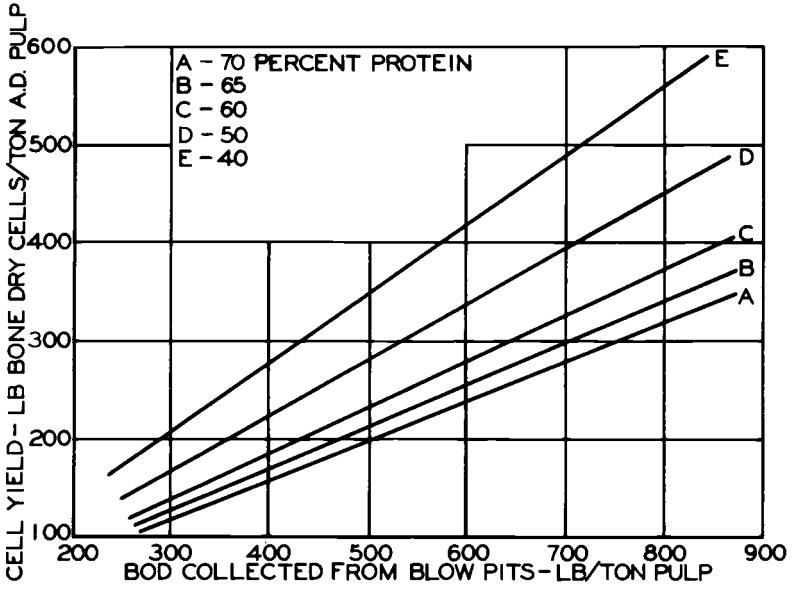

Figure 5. Relationship between cell yield, BOD collected, and protein content of cells.

Nutritive value of bacterial cells

A washed sample of cells was submitted for vitamin and amino acid assays to the Food Research Laboratories of Long Island City, New York. The sample contained 68% crude protein.

The results of the vitamin assays, Table 14, show that the cells are an excellent source of most of the essential vitamins. Table 15 shows, for comparison, the vitamin content of a number of protein concentrates used in animal feeding. The bacterial cells as a source of vitamins were superior to soybean meal, fish meal, and other oilseed meals. When compared with the bacterial cells, brewer's and Torula yeast were superior in certain of the essential vitamins.

The amino acid content of the cells is presented in Table 16. The material appeared to be complete in most of the essential amino acids, and was particularly high in phenylalanine, leucine, and valine. A comparison of the amino acid content of various protein feed concentrates, presented in Table 17, indicated the cells were a more complete ration and source of amino acids than any of the other common feed materials. The cell material was similar to casein in respect to most of the amino acids.

Table 14.
Vitamin assays of bacterial cells
(68% crude protein)

Determination	Procedure	Mg/100 gm dry wt
Thiamine	Thiochrome*	1.07
Riboflavin	Microbiological*	2.11
Pantothenic acid (total)	Microbiological*	25.09
Niacin	Microbiological*	25.28
Pyridoxine	Microbiological**	1.75
Folic acid (total)	Microbiological*	1.23
Vitamin B_{12} activity	Microbiological*	0.358
Choline	Microbiological***	54.40
Inositol	Microbiological***	181.00
Biotin	Microbiological	--

* Official Methods of Analysis of the Association of Official Agricultural Chemists, 8th edition, 1955.

** Association of Official Agricultural Chemists, No. 506, 1955.

*** Barton-Wright, E.C., The Microbiological Assay of Vitamin B-Complex and Amino Acids. New York: Pitman Publishing Corp, 1952.

Table 15.

Comparison of vitamin content of protein concentrate feeds (Mgm/lb of dry weight)

Vitamin	Bacterial cells	Torula* yeast	Brewer's** yeast	Soybean** meal	Fish** meal
Thiamin	4.86	2.41	35.0	2.5	0.45
Riboflavin	9.58	20.40	15.0	1.3	3.10
Niacin	114.80	189.00	220.0	10.0	30.00
Pantothenic acid	114.00	16.90	45.0	6.8	4.00
Pyridoxine	7.95	17.40	---	---	---
Folic acid	5.58	9.76	20.0	3.5	0.00
Vitamin B_{12} activity	1.622	---	0.0005	0.0009	---
Choline	246.50	---	---	1250	1230
Inositol	822.00	---	---	---	---
Biotin	---	---	---	---	---

* Inskeep, G.C., et al, "Food Yeast from Sulfite Liquor," Industrial and Engineering Chemistry, Vol 43, No. 8, pp 1702-1711, 1951.

** Titus, H.W., The Scientific Feeding of Chickens. Danville, Illinois: The Interstate Press, 1955.

Table 16.

Microbiological determination of amino acids as indicated

Determination	Percent of dry weight	Percent of protein
Arginine	6.14	9.02
Leucine	12.46	18.32
Isoleucine	6.67	9.81
Lysine	5.26	7.75
Methionine	1.12	1.64
Phenylalanine	21.05	30.95
Tryptophane	1.57	2.30
Histidine	2.40	3.53
Threonine	--	--
Valine	8.77	12.90

Table 17.

Comparison of amino acid content of protein feed concentrates

(Percent of dried feed)

	Protein N × 16	Argenine	Lysine	Methionine	Cystine
Cottonseed meal*	41.0	3.10	1.20	0.72	0.84
Linseed meal*	34.0	2.50	0.94	0.68	0.65
Peanut meal*	41.0	4.10	1.20	0.49	0.66
Soybean meal*	44.0	2.70	2.60	0.74	0.77
Casein*	85.0	3.50	6.70	2.70	0.28
Fish meal*	60.0	3.80	4.00	1.80	0.63
Meat scrap*	55.0	3.70	3.10	1.20	0.58
Brewer's yeast*	45.0	1.90	3.30	0.86	0.45
Torula yeast**	48.0	3.61	4.14	0.84	0.68
Bacterial cells	68.0	6.14	5.26	1.12	--

	Tryptophane	Glycine	Isoleucine	Leucine
Cottonseed meal*	0.47	2.20	1.60	2.10
Linseed meal*	0.54	--	1.40	2.10
Peanut meal*	0.39	2.30	1.40	2.40
Soybean meal*	0.55	7.10	2.20	3.20
Casein*	1.10	1.80	5.70	8.90
Fish meal*	0.69	3.30	3.20	4.90
Meat scrap*	0.35	2.50	2.30	4.70
Brewer's yeast*	0.59	--	2.60	3.20
Torula yeast**	0.66	0.22	3.75	3.57
Bacterial cells	1.57	--	6.67	12.46

	Phenylalanine	Threonine	Valine	Histidine	Tyrosene
Cottonseed meal*	2.20	1.20	1.80	1.10	1.20
Linseed meal*	1.70	1.60	1.90	0.51	1.70
Peanut meal*	2.20	0.62	2.30	0.86	1.80
Soybean meal*	2.20	1.80	2.10	1.10	1.50
Casein*	4.60	3.70	6.00	2.70	5.40
Fish meal*	2.70	2.60	3.10	1.50	2.00
Meat scrap*	2.40	2.10	2.70	1.40	1.70
Brewer's yeast*	2.00	2.30	2.40	1.20	1.30
Torula yeast**	2.41	2.58	2.98	1.31	--
Bacterial cells	21.05	--	8.77	2.40	--

* Titus, H. W., The Scientific Feeding of Chickens. Danville, Illinois: The Interstate Press, 1955.

** Inskeep, G.C., et al, "Food Yeast from Sulfite Liquor," Industrial and Engineering Chemistry, Vol 43, No. 8, pp 1702-1711, 1951.

Feeding tests

A series of rat feeding tests were conducted to determine the nutritive value of the washed bacterial cell product. The washed cells had a crude protein content of 70%. Six rats were used per ration.

The experimental rations are given in Tables 18 and 19, and the results of the growth studies are presented in Table 20.

Table 18.
Ration composition--rat growth experiments

Ration No.** / Constituent	439* %	440* %	441* %	442* %	443* %	444* %	445* %
Dried washed cells	40.0	29.0	10.0	26.0	27.5	27.5	22.0
Jones salt mixture	4.0	4.0	--	4.0	4.0	4.0	4.0
Mazola oil	3.0	3.0	--	3.0	3.0	3.0	3.0
Starch	53.0	64.0	--	65.0	64.5	64.5	66.0
Dl methionine	--	--	--	1.0	1.0	--	--
Dl isoleucine	--	--	--	1.0	--	1.0	--
Stock No. 447	--	--	90.0	--	--	--	--
Casein	--	--	--	--	--	--	5.0
Crude protein, %	27.7	20.0	20.8	20.0	20.0	20.0	20.0

* Adequate vitamin supplements were given.

** Ration Nos. 446 and 447 were stock rat rations containing about 20% crude protein.

Table 19.
Stock ration composition (No. 447)
("Breeder's mash")

Crude protein	20%
Crude fat	3
Crude fiber	8
Ash	11
NFE	58
	100%

Components: corn, oats, wheat, barley, millrun, soybean meal, fish meal, alfalfa, leaf meal, minerals, and certain vitamins

The cells fed at a 40% level of the ration supplied adequate amounts of the essential amino acids needed for rat growth. With the exception of the first week, which could be considered an acclimation period, the rats gained--as did those on either of the stock rations (446 and 447).

When the cells were fed at a 20% protein level (29% cells), certain amino acids became limiting factors in growth. When 1% dl methionine and 1% isoleucine were added (ration 442), growth was equivalent to the stock rations. When the two amino acids were added singly to the ration, the growth response was not as great. Of the two amino acids, dl methionine appeared to be the major limiting amino acid. In this respect, the bacterial cell material appeared to be quite similar to Torula yeast, which was also deficient in one or more essential amino acids.

Harris, Hajny, and Johnson (2) compared the Torula type yeast with casein as a source of protein. When the rat diet was supplemented with methionine, they found that growth characteristics were comparable with those observed when the rats were fed the casein diet. Without additional methionine, however, they reported that the gain in weight of the rats fed Torula yeast was only 60% of that for the casein.

Experiments by Fink and Hock (10) on rats have also shown that yeast proteins were not complete substitutes for animal proteins in filling the nutritional requirements. With yeast as the

only protein in the diet (except for the 15.5% always supplied from cereals in the basic diet), growth was abnormal and stunted, and death resulted from a specific type of liver (and kidney) damage. Growth remained normal, however, with 35% of the total protein supply in the form of fish and milk protein, and 50 to 60% yeast protein.

It was further shown that if the yeast protein was supplemented with cystine in amounts equal to 2% of the total protein, then brewer's yeast was a fully adequate substitute for the animal protein and maintained normal growth of the animals. In the case of Torula yeast, the addition of cystine gave marked improvements in weight and growth curves, but the normal values were not attained. The results indicated that the nutritional values of brewer's yeast and Torula were not quite the same, and that Torula (quantitatively, at least) was evidently deficient in one or more amino acids in addition to cystine.

It should be emphasized that in the feeding experiments using bacterial cells, a complete substitute of albumin was not possible unless very high concentrations of cells (40%) were used. However, the feasibility of using the bacterial cells as a partial protein supplement appeared possible. For example, the rats fed 10% cells plus the breeder's mash showed an equivalent growth response when compared with the controls.

The initial feeding experiments showed conclusively that the protein concentrate was palatable in concentrations as high as 40% of the ration, and that the material was nontoxic.

From the initial experiments it appeared reasonable to assume the cells would be a satisfactory supplement in the rations of livestock.

Table 20.

Summary of rat growth experiments on washed bacterial cells

Ration	Description	Average weight gain in grams per week percent of control					
		1st week	2d week	3d week	4th week	Total gain	Avg gain per week
439	40% cells	19.6	39.4	37.2	33.8	130.0	32.5
440	29% cells (20% protein)	17.7	23.2	21.2	28.5	90.8	22.7
441	Stock & 10% cells	32.8	39.7	34.8	31.0	138.4	34.6
442	20% cells, 1% each of dl methionine and dl isoleucine (20% protein)	24.6	45.2	35.0	41.8	146.8	36.7
443	27.5% cells, 1% dl methionine (20% protein)	20.3	35.2	26.0	32.5	114.0	28.5
444	27.5% cells, 1% dl isoleucine (20% protein)	5.5	22.4	30.0	25.6	83.6	20.9
445	22% cells & 5% casein	16.0	34.4	25.8	29.0	105.2	26.3
446	Stock, type B	33.3	42.0	35.8	33.8	144.8	36.2
447	Stock, type A	33.7	41.0	35.0	33.8	143.6	35.9

Market potential

The largest market for the bacterial cells would be as a high protein supplement if feeding and price conditions were satisfactory. Schleef (8) estimated that in 1947 approximately 364,000 tons of oilseed meals were consumed by the livestock of the Pacific Coast area. On the basis of oilseed meal now used by different types of livestock, it would appear that the poultry, dairy, and hog industries (in descending order), would furnish the largest potential market for competitive protein.

If the entire sulfite industry of the Pacific Northwest would produce high protein concentrate, only 30% of the Pacific Coast demand for protein would be satisfied. It would seem, therefore, that if the cell material could be produced at a competitive cost, an adequate market would certainly be available.

Since most of the protein now used must be transported from the Midwest, producers of protein concentrate in the Pacific Northwest would always be ensured of a minimum freight differential of at least $25 per ton. By increasing the protein content to 65 to 70%, the freight differential would be much higher.

Soybean meal is the most important vegetable-protein concentrate fed to animals on the Pacific Coast, and is selling at the present time at about $85 per ton (44% protein). Since this material is sold on a nitrogen basis, the bacterial cells at an average protein content of 65% could then sell at $65/44 \times \$85$, or $126 per ton.

Plant layout and design

Layout and design of a treatment plant to effect 80% BOD reduction and produce a protein concentrate are influenced by numerous factors, which will vary from mill to mill. In most mills undertaking a major pollution abatement program, the collection system would require redesign for the effective collection of solids. The collection of 70 to 80% of the solids could be accomplished effectively by the conventional blow-pit washing, but to collect 90% or more of the solids at a relatively high solids

concentration would require rotary vacuum washers or screwpress washers. The minimum expenditure for a 200-ton sulfite mill for collection system improvements would probably amount to about $100,000 (70 to 80% collection of solids). Screening of the spent liquor ahead of the SO_2 strippers would be required to remove fibers.

Although steam stripping of raw waste is not necessary when using waste up to 8.0% total solids, steam stripping would be desirable because the neutralization requirements could be cut in half.

The type of neutralizing agent utilized depends upon the liquor used and the subsequent use of the treated effluent. For example, when using CaO base liquor, the addition of ammonia as a nitrogen source would be more than adequate to maintain pH at neutrality. Lime would be the cheapest neutralizing agent when using ammonia base liquor, but additional equipment would be required to remove the precipitate resulting from lime addition. Lime also would increase the scaling problem if the effluent was to be further processed. Under most conditions, anhydrous ammonia would be the best neutralizing agent.

The aeration of liquor in the presence of cells could be conducted in conventional sewage treatment tanks. Considerable saving in space could be effected employing the Currie claraerator because the aeration channels and thickener are incorporated in a single design.

It has been calculated that a 200-ton sulfite mill could treat its spent sulfite liquor in a claraerator having an overall diameter of 110 feet, thickener diameter of 82 feet, and the outer annular aeration channel a width of 14 feet with side water depth of 15 feet. The use of the thickener would reduce the volume of cells to the primary centrifuges by 50%, and would effect a considerable saving in centrifugal equipment and power.

Table 21 presents the dimensions of the aeration and thickening units required for various pulp mill capacities.

Table 21.
Size of aeration and thickening tanks required
to treat spent sulfite liquor to 80% BOD reduction
(BOD load = 2600 lb/1000 cu ft/day)

Pulp capacity tons/day	Total unit diameter ft	Thickener		Aeration channel	
		Diameter ft	SWD depth ft	Width ft	SWD ft
100	78	50	12	14	15
200	110	82	12	14	15
300	136	100	12	18	15

The thickened cells having a cell concentration of 4% and a total solids content of about 13%, would then pass to the primary centrifuges where the material would be thickened further. The cells are then diluted with water to about a 1% cell suspension, and recentrifuged. About two to three washing cycles will be required to remove the spent liquor from the cells.

The final washed cells, having an average protein content of about 66% and a total solids content of about 15%, would be dried on drum driers. The use of vacuum filters for the removal of water prior to drying would be extremely difficult because of the gelatinous character of the cell material. Clogging of the filter cloth would occur quite rapidly.

In Germany, evaporation of yeast cells to 30% solids was employed, followed by drum driers or spray driers. Spray drying of the solids was somewhat cheaper than drum drying, but the spray dried product was not as palatable as the drum dried product. In practice, the cells would be dried to about 7 to 10% moisture and then bagged.

A schematic diagram of the total plant is shown in Figure 6.

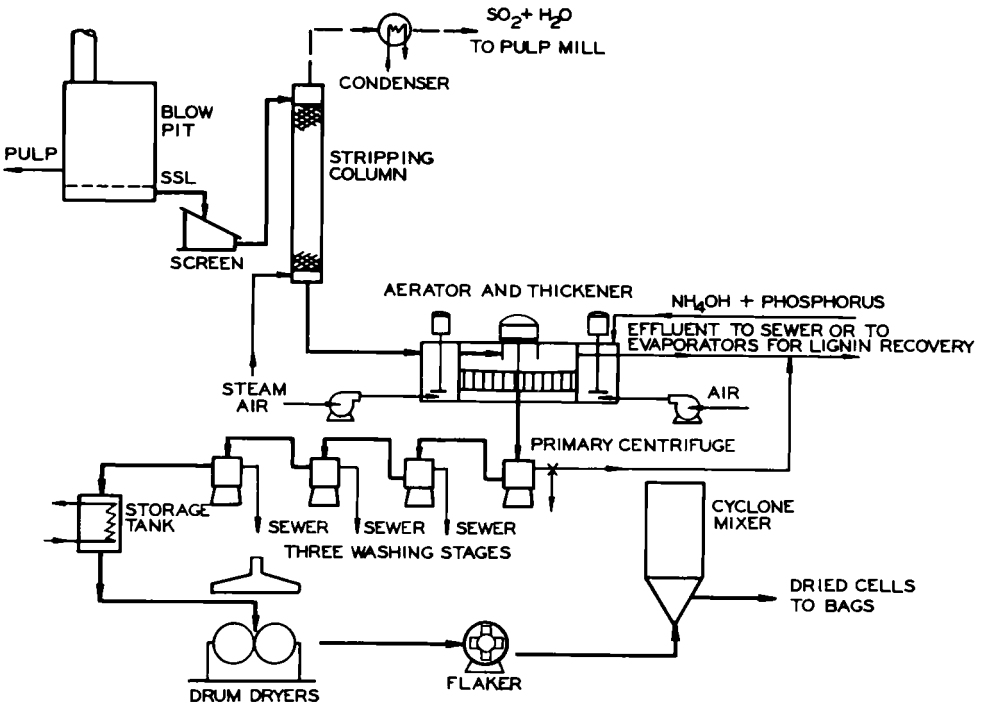

Figure 6. Flow sheet for production of protein concentrate.

Production cost

Because the production of bacterial protein was a new process, no production costs were available from which an exact cost determination could be made. However, the process paralleled very closely those employed in the compressed yeast, Torula yeast, and activated sludge fermentations.

Ross, et al (6) conducted a comprehensive survey on the production of Torula yeast from spent sulfite liquor and found that yeast containing 48 to 50% protein could be produced to sell profitably at about $140 per ton for a 100-ton sulfite mill. The author estimated the cost of erecting a yeast plant producing 2500 tons of yeast per year would be $600,000. This figure also included equipment for collection of spent sulfite liquor.

To arrive at an approximate cost for producing bacterial protein, preliminary cost estimates were made on the basis of a 100-ton pulp mill. Yearly production of bacterial cells was calculated at 3600 tons for 360 operating days. The estimated cost of the plant was $400,000. The cost of collection system improvements or vacuum washers was not included in the cost estimate. The operating costs per ton of product, presented in Table 22, were $55.62 when fermenting ammonia base liquor. The chemical costs for the fermentation of calcium, magnesium, or sodium spent sulfite liquor were $25.54, compared with $17.84 for the ammonia base liquor. The operational cost of producing bacterial protein from bases other than ammonia would be $63.32 per ton of product.

The total production cost, profit, and pay-out time are presented in Table 23. The total cost of producing bacterial cells containing 65% protein would be $81.20 for ammonia base liquor, or $88.90 for calcium base liquor. At a sales cost of $0.06 per pound, profit after taxes would be $18.60 per ton of product (ammonia base liquor), which would require six years for pay-out time.

Table 22.

Operating costs for production of 66% bacterial protein from ammonia base spent sulfite liquor

Plant size: 100 tons ammonia base sulfite pulp per day
3600 tons of cells per year (360 operating days)

		Dollars per ton of product
Chemical costs		
Phosphorus	$10.44	
Neutralization	7.40	17.84
Labor		
(Wages $2.33/hr)		
3 men for 1 shift		
2 men for 2 shifts		13.10
Water		
($7/million gallons)		2.82
Power		
($0.005/kwhr)		
Air and mixers	8.05	
Miscellaneous	1.21	9.26
Steam		
($0.50/1000 lb)		
271,800 lb/day		13.60
Bags for wrapping		
($0.10/50-lb bag)		4.00
Total		60.62
Less chemical saving of sulfur from steam stripper		5.00
Total operating costs		55.62

Table 23.

Total product cost, profit, and pay-out time for production of 66% bacterial protein from ammonia base spent sulfite liquor

		Dollars per ton of product
Total product cost		
Operating cost	$55.62	
Capital charges	11.12	
Maintenance charges	8.90	
Mill burden cost	5.56	
Total cost		81.20
Sales receipts		
At 100% capacity, 10 tons/day, 360 operating days at $0.06/lb		120.00
Less cost		81.20
Profit before taxes		38.80
Federal taxes		
Less 52%		20.20
Profit after taxes		18.60
Payment time in years		
400,000/67,000		5.97

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